In the legends of figures 1-14, 5 observations represent 3 human subjects or 6 rats and for figures 16 and 18, three separate experiments denote 3 human subjects or 6 rats.

Range of serum hormones (FSH, LH, testosterone and estradiol) of the subjects selected for the study (Table 1)

Serum FSH, LH, testosterone and estradiol showed an age-matched normal ranges suggesting the normal testicular function and regulation in the selected subjects.

Dose-dependent effects of gamma radiation on LH receptor concentration in cultured human and rat Leydig cells (Figures 1 & 2)

Both human and rat Leydig cell surface LH/hCG receptor concentrations were significantly decreased following higher doses of radiation exposure (6 Gy and above), while lower doses of radiation (2 and 4 Gy) had no influence on the receptors.

Dose-dependent effects of gamma radiation on basal and LH-stimulated cAMP production in cultured human and rat Leydig cells (Figures 3 & 4)

Gamma radiation was found to have an inhibitory effect on both basal and LH-stimulated cAMP production in a dose-dependent manner in both human and rat Leydig cells. Lower doses of radiation (2 and 4 Gy) did not induce any change but higher doses of radiation exposure (6 Gy and above) brought down both basal and LH-stimulated cAMP production. Human Leydig cells produced more amount of cAMP under basal and LH-stimulated conditions than rat Leydig cells. LH failed to stimulate cAMP production
Table 1: Range of serum hormones (FSH, LH, Testosterone and Estradiol) of the subjects selected for the study

<table>
<thead>
<tr>
<th>Age</th>
<th>FSH (IU/L)</th>
<th>LH (IU/L)</th>
<th>Testosterone (ng/ml)</th>
<th>Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-70 years</td>
<td>12 - 17</td>
<td>15 - 19</td>
<td>3.5 - 7.4</td>
<td>55 - 74</td>
</tr>
</tbody>
</table>

Number of Patients = 30
Fig. 1 Dose-dependent effects of gamma radiation on LH/hCG receptor concentration in human Leydig cells *in vitro*

Each bar represents the mean ± SEM of 5 observations. Significance at *p<0.05.*

a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy.
Fig. 2  Dose-dependent effects of gamma radiation on LH/hCG receptor concentration in rat Leydig cells *in vitro*

Each bar represents the mean ± SEM of 5 observations. Significance at $p<0.05$. a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy.
Fig. 3  Dose-dependent effects of gamma radiation on basal and LH-stimulated cAMP production by human Leydig cells in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at p < 0.05.
* - compared with basal; a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy.
Fig. 4  Dose-dependent effects of gamma radiation on basal and LH-stimulated cAMP production by rat Leydig cells in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at $p<0.05$.  * - compared with basal; a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy.
following exposure to 4 Gy and above doses of radiation in human Leydig cells. However, rat Leydig cells responded to LH stimulation even after higher doses of radiation exposure but the magnitude of stimulation was decreased in a dose-dependent manner.

Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated cytochrome P₄₅₀ cholesterol side chain cleavage (P₄₅₀sec) enzyme activity in cultured human and rat Leydig cells (Figures 5 & 6)

Cyclic AMP stimulated the P₄₅₀sec enzyme activity more effectively than LH in control as well as irradiated Leydig cells of human and rat. Higher doses (6, 8 and 10 Gy) of radiation decreased the enzyme activity under basal and stimulated conditions in a dose-dependent manner. However, low doses did not induce any appreciable change in the enzyme activity.

Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme activity in cultured human and rat Leydig cells (Figures 7 & 8)

Radiation exposure diminished 3β-HSD enzyme activity in a dose-dependent fashion in both human and rat Leydig cells. While higher doses (6, 8 and 10 Gy) of radiation decreased the enzyme activity under basal and stimulated conditions, lower doses did not induce any appreciable change. Cyclic AMP stimulated the enzyme activity more effectively than LH in both control and irradiated Leydig cells.
Fig. 5 Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated P₄₅₀ side chain cleavage enzyme activity in human Leydig cells in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at p<0.05. a - compared with Control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy; 1 - compared with basal; 2 - compared with LH-stimulated.
Fig. 6 Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated P_{450} side chain cleavage enzyme activity in rat Leydig cells in vitro.

Each bar represents the mean ± SEM of 5 observations. Significance at $p<0.05$. 
- a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; 
- d - compared with 6 Gy; e - compared with 8 Gy; 1 - compared with basal; 
- 2 - compared with LH-stimulated.
Fig. 7  Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated 3β-HSD activity in human Leydig cells in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at p<0.05.  
a - compared with control;  
b - compared with 2 Gy;  
c - compared with 4 Gy;  
d - compared with 6 Gy;  
e - compared with 8 Gy;  
1 - compared with basal;  
2 - compared with LH-stimulated.
Fig. 8  Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated 3β-HSD activity in rat Leydig cells in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at $P<0.05$. a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy; 1 - compared with basal; 2 - compared with LH-stimulated.
Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated 17β-hydroxysteroid dehydrogenase (17β-HSD) enzyme activity in cultured human and rat Leydig cells (Figures 9 & 10)

17β-HSD enzyme activity was decreased in a dose dependent manner following higher doses of radiation exposure. While lower doses (2 & 4 Gy) had no effect on the enzyme activity under basal and stimulated states in both human and rat Leydig cells, higher doses (6, 8 and 10 Gy) of radiation decreased the enzyme activity significantly. As in the case of other steroidogenic enzymes, cAMP stimulated the 17β-HSD activity more effectively than LH.

Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated testosterone production by cultured human and rat Leydig cells (Figures 11 & 12)

Human Leydig cells produced more amount of testosterone than rat Leydig cells under basal and stimulated conditions. Cyclic AMP stimulated the testosterone production effectively than LH in control and irradiated human and rat Leydig cells. While lower doses (2 and 4 Gy) of radiation increased the basal testosterone production slightly in rat Leydig cells, the same doses did not affect the basal testosterone production by human Leydig cells. Radiation was found to inhibit human and rat Leydig cell testosterone production in a dose-dependent manner. Lower doses did not exert any
Fig. 9  Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated 17β-HSD activity in human Leydig cells

*in vitro*

Each bar represents the mean ± SEM of 5 observations. Significance at $p<0.05$.  

- a - compared with control;  
- b - compared with 2 Gy;  
- c - compared with 4 Gy;  
- d - compared with 6 Gy;  
- e - compared with 8 Gy;  
- 1 - compared with basal;  
- 2 - compared with LH-stimulated.
Fig. 10  Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated 17β-HSD activity in rat Leydig cells 
in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at $p<0.05$.  
- a - compared with control;  
- b - compared with 2 Gy;  
- c - compared with 4 Gy;  
- d - compared with 6 Gy;  
- e - compared with 8 Gy;  
- 1 - compared with basal;  
- 2 - compared with LH-stimulated.
Fig. 11  Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated testosterone production by human Leydig cells in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at *p*<0.05. a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy; 1 - compared with basal; 2 - compared with LH-stimulated.
Fig. 12  Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated testosterone production by rat Leydig cells in vitro

Each bar represents the mean ± SEM of 5 observations. Significance at p<0.05. a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy; 1 - compared with basal; 2 - compared with LH-stimulated.
adverse effect on rat and human Leydig cell testosterone production under basal and stimulated conditions. However, higher doses of radiation (6 Gy and above) decreased the same. The adverse effect of radiation was more pronounced with increasing doses of radiation.

Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated estradiol production by cultured human and rat Leydig cells (Figures 13 & 14)

Human Leydig cells produced more amount of estradiol than rat Leydig cells under basal and stimulated conditions. Radiation exposure depressed the Leydig cell estradiol production in a dose-dependent fashion similar to that of testosterone under basal and stimulated conditions in human and rat Leydig cells. As in the case of steroidogenic enzymes and testosterone production, cAMP-induced estradiol production was more when compared to LH in human and rat Leydig cells.

Dose-dependent effects of gamma radiation on LH receptor mRNA expression in cultured human and rat Leydig cells (Figures 15 – 18)

LH receptor mRNA expression was found to be increased following 2 Gy of radiation exposure in human Leydig cells, while other doses tested had no influence on the same. However, in rat Leydig cells LH receptor mRNA expression was not altered under any of the doses employed.
Fig. 13 Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated estradiol production by human Leydig cells *in vitro*

Each bar represents the mean ± SEM of 5 observations. Significance at $p<0.05$. a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy; 1 - compared with basal; 2 - compared with LH-stimulated.
Fig. 14  Dose-dependent effects of gamma radiation on basal, LH and cAMP-stimulated estradiol production by rat Leydig cells 

*in vitro*

Each bar represents the mean ± SEM of 5 observations. Significance at p<0.05. a - compared with control; b - compared with 2 Gy; c - compared with 4 Gy; d - compared with 6 Gy; e - compared with 8 Gy; 1 - compared with basal;  2 - compared with LH-stimulated.
Fig. 15 Agarose gel electrophoresis of RT-PCR products showing the effects of gamma radiation on LH receptor mRNA expression in human Leydig cells *in vitro*

Lane 1: Molecular marker (100 bp ladder)
Lane 2: Control
Lane 3: 2 Gy treated
Lane 4: 4 Gy treated
Lane 5: 6 Gy treated
Lane 6: 8 Gy treated
Lane 7: 10 Gy treated
Fig. 16  Dose-dependent effects of gamma radiation on LH receptor mRNA expression in human Leydig cells in vitro

Each bar represents the mean ± SEM of three separate experiments. Significance at $p<0.05$. * - compared with control.
Fig. 17  Agarose gel electrophoresis of RT-PCR products showing the effects of gamma radiation on LH receptor mRNA expression in rat Leydig cells *in vitro*

Lane 1: Molecular marker (100 bp ladder)
Lane 2: Control
Lane 3: 2 Gy treated
Lane 4: 4 Gy treated
Lane 5: 6 Gy treated
Lane 6: 8 Gy treated
Lane 7: 10 Gy treated
Fig. 18  Dose-dependent effects of gamma radiation on LH receptor mRNA expression in rat Leydig cells in vitro

Each bar represents the mean ± SEM of three separate experiments.